Chemical and Enzymatic Investigation of the Leaf Cuticle of Pear Genotypes Differing in Resistance to Pear Psylla

Hervé C. Gérard,* William F. Fett, Robert A. Moreau, Stanley F. Osman, and Robert L. Miller

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane, Philadelphia, Pennsylvania 19118

The composition and structure of dewaxed cuticular membranes of three genotypes of pear (*Pyrus* spp.) leaves differing in resistance to attack by pear psylla (*Cacopsylla pyricola* Forester) were studied by chemical and enzymatic hydrolysis. The amount of chloroform/methanol-soluble material obtained after hydrolysis by methanolic KOH (indicative of leaf cuticle thickness) was inversely proportional to the relative degree of insect resistance. HPLC and GC/MS analyses were used to identify and quantify the fatty acids and phenolics present in the extracts. The major cutin monomer of all three genotypes was 10,16-dihydroxyhexadecanoic acid. Enzymatic hydrolysis with three commercial lipases and a cutinase isolated from *Nectria haematococca* indicated that there were structural differences among the dewaxed cuticular membranes of the pear genotypes.

INTRODUCTION

Cacopsylla pyricola Forester (pear psylla) infests pear leaves and is one of the most destructive insect pests of pear in the United States and Europe (Butt and Stuart, 1985). Pear genotypes differ in their susceptibility to attack by this parasite (Schalk and Ratcliffe, 1976). Volatile compounds given off by leaves of Bartlett (susceptible) and Bradford (resistant) were recently compared in our laboratory, but no gross differences related to their relative susceptibility to pear psylla were found (Miller et al., 1989). In addition to olfactory responses, previous investigations of passive insect resistance mechanisms have identified structural and chemical components that affect feeding behavior of phytophagous insects [for reviews see Chapman and Bernays (1989) and Woodhead and Chapman (1986)]. Trichomes may prevent insect feeding, and trichome exudates may be toxic. The plant cuticle is another barrier to insect feeding that must be overcome. The plant cuticle or cuticular membrane is comprised of two components. The structural component, cutin, is an insoluble polyester, with the major monomers being C_{16} and C_{18} hydroxy and hydroxyepoxy fatty acids (Holloway, 1982; Kollatukudy, 1987). The cutin is embedded in cuticular wax and is covered by epicuticular wax, both of which are made up of chloroform/methanol-soluble lipids such as hydrocarbons and wax esters (Baker, 1982). Several studies have demonstrated that common components of epicuticular waxes such as alkanes, fatty alcohols, and fatty acids (Baker, 1982) can be phagostimulatory or possess deterrent activity. Recently, Eigenbrode et al. (1991) reported significant differences in the composition of epicuticular lipids between cabbage cultivars resistant or susceptible to the diamondback moth. Yang et al. (1993) also found a relationship of the cuticular lipid composition of wild and domesticated peanuts to their relative resistance to fall armyworm and thrips. In addition to nonpolar compounds, polar compounds present at the leaf surface may also affect feeding. For instance, when incorporated into parafilm sachets, the phenolic glycoside phlorizin obtained from the surface of apple leaves stimulated probing by two apple aphids but inhibited probing by a third aphid which does not attack apple (Klingauf, 1971). In fact, Chapman and Bernays (1989) concluded that secondary plant compounds are feeding deterrents for nonhost plants for most, if not all, phytophagous insects. Other possible factors affecting insect feeding behavior are leaf cuticle thickness and three-dimensional structure.

The objectives of this study were to compare the cutin monomer composition and cuticular structure of pear leaf cuticular membranes of three pear genotypes using both chemical and enzymatic hydrolysis. To our knowledge this is the first report on the cutin monomer composition of pear leaf cutin and the first to attempt to relate insect resistance to cuticular membrane structure by use of enzymatic hydrolysis.

EXPERIMENTAL PROCEDURES

Chemicals. All organic solvents used were of HPLC grade purchased from Burdick and Jackson (Muskegon, MI). Phenolic standards (*p*-coumaric, ferulic, and vanillic acids), ω-hydroxyhexadecanoic acid, cellulase (EC 3.2.1.4), pectinase (EC 3.2.1.15) from *Aspergillus niger*, and lipases from a *Pseudomonas* species (Type XIII) and *Candida cylindracea* (Type VII-S) were obtained from Sigma Chemical Co. (St. Louis, MO). Lipase PS-800 (produced by a *Pseudomonas* spp.) was a gift from Amano International Enzyme Co., Inc. (Troy, VA).

Isolation of Pear Leaf Dewaxed Cuticular Membranes. Pear leaves from single trees of Bartlett (*Pyrus communis*) and NY 10355 (breeding selection, *P. communis* × *Pyrus ussuriensis*) as well as from two separate trees (in the same orchard) of Erabasma (P. I. 48370, *P. communis* × unknown *Pyrus* spp.) grown at the USDA, ARS, Appalachian Fruit Research Center (Kearneysville, WV) were kindly supplied by Dr. R. L. Bell. Bartlett is susceptible, NY 10355 is resistant (Butt et al., 1988), and Erabasma is intermediate (Bell and Stuart, 1990) in resistance to attack by pear psylla. Pear leaf cuticular membranes were prepared as previously described (Baker and Procopiou, 1975) with minor modifications. Leaf disks (3 cm^2, one per leaf) were removed and washed in chloroform for 3 min to remove epicuticular wax and then incubated in a clarified solution of pectinase (0.5 g/L) and cellulase (2.5 g/L) in 0.05 M citrate–phosphate buffer, pH 4.0, at 28 °C for 14 h. Finally, the cuticular membranes were washed sequentially in chloroform, chloroform/methanol (2:1), and methanol to remove cuticular wax, pigments, or other lipids.

Cutinase from *Nectria haematococca*. *N. haematococca* (*Fusarium solani* f. sp. *pisi*) strain T8 was kindly supplied by Dr. H. D. VanEtten. The fungus was grown on potato dextrose agar (Difco Laboratories, Detroit, MI) and used to inoculate a basal mineral medium supplemented with apple cutin (0.4% w/v) as

Table 1. Composition (Milligrams per Gram of Total Cutin Monomers) of Dewaxed Pear Leaf Cuticular Membranes

	genotype			
		Erabasma[b]		
monomers[a]	Bartlett	tree 1	tree 2	NY 10355
(A) hexa- and octadecanoic acids	245.0[c]	149.0	290.0	142.0
(B) vanillic acid	2.2	2.3	2.2	1.8
(C) *p*-coumaric acid	0.4	0.4	0.2	0.2
(D) ferulic acid	2.3	1.6	1.1	1.5
(E) 18-hydroxyoctadeca-9-enoic acid	9.5	2.5	2.5	7.0
(F) 16-hydroxyhexadecanoic acid	7.5	5.0	5.0	17.0
(G) 18-hydroxy-9,10-epoxyoctadecanoic acid	22.3	73.0	60.9	36.0
(H) unidentified monomer	72.0	73.0	30.0	32.0
(I) 9,16-dihydroxyhexadecanoic acid	54.5	41.0	60.0	73.0
(J) 10,16-dihydroxyhexadecanoic acid	594.0	640.0	527.0	676.0
(K) 9,10,18-trihydroxyoctadecanoic acid	9.5	12.0	13.0	8.0
totals	1009.2	998.8	991.9	994.5
% phenolics[d]	0.48	0.43	0.37	0.35

[a] Listed in order of elution. [b] Leaves were collected from two trees in the same orchard. [c] Values for phenolics and fatty acids are averages of data obtained from five experiments (10 membranes total). Coefficients of variation were usually less than 5%. [d] Total phenolics/(total phenolics + total fatty acids) × 100.

previously described (Hankin and Kolattukudy, 1968). The extracellular fluid was collected by filtration and lyophilized, and the cutinase was partially purified by precipitation with ammonium sulfate at 50% of saturation. The precipitate was collected, dissolved in 50 mM sodium phosphate buffer, pH 7.0, and dialyzed against the same buffer as previously described (Kolattukudy et al., 1981). Protein content was determined according to the method of Bradford (1976).

Chemical and Enzymatic Hydrolysis. Chemical hydrolysis was performed by treating two dewaxed cuticular membranes with 1 mL of 1.5 N KOH dissolved in methanol plus 200 μL of deionized water at 28 °C for 18 h. The resulting solution was acidified with acetic acid, and released fatty acids and phenolic components were extracted with chloroform/methanol (Bligh and Dyer, 1959). Organic phases were removed and evaporated to dryness under nitrogen. The residue was weighed, dissolved in 1 mL of chloroform/methanol (85/15), and filtered through glass wool into HPLC autosampler vials. A second KOH hydrolysis of the residue yielded no further extractable materials. The average recoveries of standard phenolics and hydroxy fatty acids added at the start of the hydrolysis procedure instead of cuticular membranes ranged between 80% and 95%.

Enzymatic hydrolyses were performed on 10 cuticular membranes in 10 mL of the appropriate buffer (0.1 M glycine–NaOH buffer, pH 9.0, for cutinase assays and 0.1 M phosphate buffer, pH 8.0, for lipases assays) and either 0.05 mg/mL of cutinase or 0.5 mg/mL of commercial lipase with shaking of 120 rpm at 28 °C for 16 h. After incubation, cutin monomers released were extracted with chloroform/methanol (Bligh and Dyer, 1959), weighed, and redissolved as described above.

Quantification and Identification of Chloroform/Methanol-Soluble Materials. *HPLC-ELSD.* Separations were performed with a Hewlett-Packard Model 1050 HPLC (Waldbroom, Germany) equipped with an autosampler connected to a Waters 350 spectrophotometer (Milford, MA) set at 280 nm for the detection of phenolic components and to a Varex Universal evaporative light-scattering detector (ELSD) (Rockville, MD) for the detection of hydroxy fatty acids as previously described (Gerard et al., 1992a). The column used was a Chrompack ChromSep 7-μm Lichrosorb Si 60 silica cartridge system (10 cm × 3 mm) (Raritan, NJ) with a flow rate of 0.5 mL/min. A binary gradient (hexane/2-propanol/acetic acid) was used for elution as previously described (Gerard et al., 1992b).

Commercial standards of phenolics and ω-hydroxyhexadecanoic acid or cutin monomers previously purified by HPLC were used to construct calibration curves representing peak area vs monomer mass. Calibration curves were linear for all three phenolic standards up to 3 μg per injection and also were linear up to 25 μg per injection for the hydroxy fatty acids. Cutin content was assessed using a standard curve of area unit vs mass of 10,-16-dihydroxyhexadecanoic acid, which constituted approximately 65% by weight of the total monomers in our pear leaf membranes as determined by alkaline hydrolysis.

Gas Chromatography/Mass Spectrometry. Isolated peaks collected off the HPLC or crude fractions were methyl esterified by treatment with boron trifluoride in methanol and then silylated with *N,O*-bis(trimethylsilyl)acetamide for 30 min at room temperature. GC/MS (electron impact) analyses were performed with a Hewlett-Packard Model 5990B instrument fitted with an Ultra 1 (methyl silicone) 12-M capillary column with temperature programming from 150 to 250 °C at 4 °C/min as previously described (Gerard et al., 1992b). Cutin monomers were identified using reference compounds and published spectra (Holloway, 1982).

RESULTS AND DISCUSSION

Chemical Hydrolysis. Resistance to pear psylla was inversely related to the mass of chloroform/methanol-soluble materials (cutin) released after chemical hydrolysis of cuticular membranes per square centimeter of leaf area. The most resistant genotype (NY 10355) had 55.0 ± 0.9 μg/cm^2 leaf area, while leaves from the two trees of Erabasma with intermediate susceptibility had 160.9 ± 3.2 (tree 1) and 180.2 ± 3.3 μg/cm^2 (tree 2). The most susceptible genotype, Bartlett, had 242.0 ± 4.8 μg/cm^2 leaf area. The values corresponded to approximately 6% (NY 10355), 12% (Erabasma), and 14% (Bartlett) of the initial membrane weights. As cutin content is positively correlated with the thickness of the cuticular membrane (Holloway, 1982), leaves of NY 10355 have thinner membranes than do leaves of the two more susceptible genotypes.

Qualitatively, the chloroform/methanol-soluble monomer compositions after base hydrolysis of the cuticular membranes were similar for the three genotypes. The major monomer present with 10,16-dihydroxyhexadecanoic acid, a common component of plant cutins (Holloway, 1982), which comprised from 56% to 68% of the totals (Table 1). This dihydroxy fatty acid was reported to be the major cutin monomer of pear fruit cutin, comprising 45% of the total monomers (Walton and Kollatukudy, 1972). In contrast to the fruit, pear leaf cutin contained 18-hydroxy-9,10-epoxyoctadecanoic acid but no 18-hydroxy-9,10-epoxyoctadec-12-enoic acid (Table 1), which comprises 25% of the total cutin monomers of pear fruit cutin (Walton and Kollatukudy, 1972). Cuticular membranes of resistant genotype NY 10355 were higher in the amounts of 16-hydroxyhexadecanoic acid than were the membranes of the other two genotypes. The leaf cuticular membranes contained considerable amounts of hexa- and octadecanoic acids (Table 1). These monoba-

Table 2. Release of Cutin Monomers by Reaction of Dewaxed Pear Leaf Cuticular Membranes with Methanolic KOH, Lipases, and Cutinase

	release of cutin monomers[a]					
	Bartlett		Erabasma (tree 1)		NY 10355	
treatment	TR[b]	HFA[c]	TR	HFA	TR	HFA
methanolic KOH	3.75 ± 0.04	1.45 ± 0.03	2.90 ± 0.18	1.05 ± 0.02	1.25 ± 0.08	0.35 ± 0.01
lipases						
C. cylindracea	0.030 ± 0.005	0	0.020 ± 0.005	0	0.030 ± 0.005	0
Pseudomonas spp.	0.080 ± 0.007	0.057 ± 0.005	0.055 ± 0.005	0.030 ± 0.005	0.020 ± 0.005	0
PS-800	0.100 ± 0.010	0.080 ± 0.007	0.080 ± 0.007	0.050 ± 0.005	0.050 ± 0.005	0
cutinase						
N. haematococca	0.350 ± 0.020	0.295 ± 0.015	0.330 ± 0.020	0.210 ± 0.010	0.120 ± 0.010	0.075 ± 0.005

[a] Experiments were repeated three times for a total of six membranes for chemical hydrolysis and 30 membranes for each enzymatic hydrolysis. [b] TR, total residue. Results are expressed as milligrams of lipid extracted per disk ± SD. [c] HFA, hydroxy fatty acids. Results are expressed as the mean milligrams of HFA released ± SD using 10,16-dihydroxyhexadecanoic as standard.

sic acids are normally minor components of plant cutins (Holoway, 1982) but are common components of cuticular waxes (Baker, 1982), indicating that not all cuticular wax components or cytoplasmic membrane lipids were removed during preparation of the membranes. Also present were lesser amounts of monohydroxy, epoxyhydroxy, and trihydroxy acids (Table 1), all typical cutin monomers (Holloway, 1982). The variability of the cutin composition that can be encountered from one tree to another is illustrated for the genotype Erabasma in Table 1.

The addition of a UV detector to the ELSD allowed us to quantify phenolics in addition to fatty acids. Figure 1 shows typical UV and ELSD chromatograms of the chloroform/methanol-soluble products after base hydrolysis of a pear cuticular membrane. The three peaks with retention times of 11.0, 13.0, and 15.0 min cochromatographed with standards of vanillic acid, ferulic acid, and coumaric acid, respectively. Absorbance values at 280 nm were used for quantification. Vanilic, *p*-coumaric, and ferulic acids were present in low amounts for all three genotypes of pear (Table 1). The levels of ferulic acid were higher and the levels of *p*-coumaric acid lower than those reported for pear fruit cutin, where they may be covalently bound (Riley and Kollatukudy, 1975). Vanillic acid was not reported to be present in pear fruit cutin (Riley and Kollatukudy, 1975), but alcoholic esters of vanillic acid were reported to occur in leaves of *Pyrus calleryana* (Challice and Williams, 1968). Recently, ferulic and *p*-coumaric acids present in cuticular lipids were reported to play a role in resistance of corn to maize weevil (Classen et al., 1990) and may be a factor in resistance of peanut species to fall armyworm and thrips (Yang et al., 1991).

Enzymatic Hydrolysis. Even though the leaf cuticular membrane compositions of chloroform/methanol-soluble materials were similar for the three genotypes of pear leaves, structural differences might exist. To detect such differences, cuticular membranes were subjected to hydrolysis with lipases and a cutinase, and the release of typical cutin monomers was determined. Lipases (glycerol ester hydrolases; EC 3.1.1.3) are enzymes that cleave ester bonds of insoluble esters at the oil–water interface (Gilbert, 1993). We have previously reported that some of the ester bonds present in cutin are hydrolyzed by certain lipases (Gerard et al., 1993).

The three commercial lipases used in this study, one from a yeast, *C. cylindracea*, and the other two from bacteria, *Pseudomonas* spp., were previously demonstrated in our laboratory to hydrolyze apple fruit cutin (Gerard et al., 1993). The lipases differed in their ability to release hydroxy fatty acids and phenolics from the cuticular membranes of the three genotypes (Table 2). Treatment with the *Candida* lipase did not cause the release of

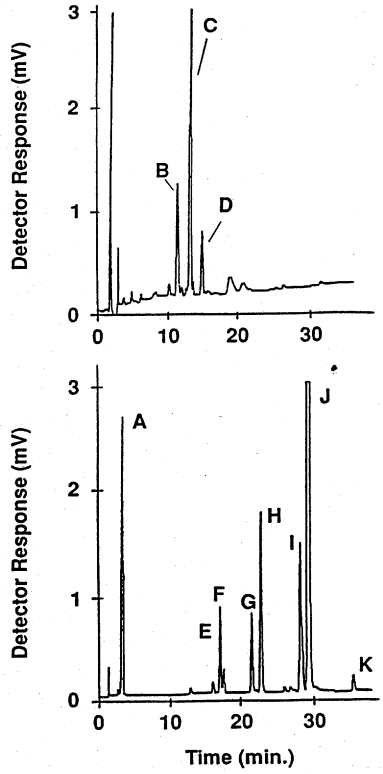

Figure 1. UV (a, top) and ELSD (b, bottom) chromatogram of a KOH hydrolysate of a NY 10355 pear leaf cuticular membrane. Lettered peaks are identified in Table 1, and labels are split between the top and bottom panels.

hydroxy fatty acids from cutins of any of the three genotypes and led to the release of phenolics (*p*-coumaric and ferulic acids) only for the pear psylla resistant genotype NY 10355 (data not shown). Both of the *Pseudomonas* lipases caused release of hydroxy fatty acids (primarily 10,16-dihydroxyhexadecanoic acid) from Bartlett and Erabasma (tree 1), but none of the lipases tested were active in releasing hydroxy fatty acids from the cuticular membranes of NY 10355. Treatment with PS-800 lipase caused the release of phenolic components from cuticular membranes of all three genotypes, while the *Pseudomonas* spp. lipase was inactive in this regard (data not shown). No qualitative differences in the types of hydroxy fatty acids and phenolics liberated by the lipases were found.

The identity and the relative amounts of monomers released from the cuticular membranes of the three genotypes treated with cutinase from *N. haematococca* strain T8 are shown in Table 3. The degree of hydrolysis with the cutinase (20% by weight) was much higher than that obtained with the lipases (5%). For Bartlett, an unknown cutin monomer (retention time = 23 min) and

Table 3. Cutin Monomers Released by Reaction of Dewaxed Pear Leaf Cuticular Membranes with Cutinase

	relative % of released fatty acids		
	Bartlett	Erabasma	NY 10355
hexa- and octadecanoic acids	13.1 ± 0.2[a]	7.2 ± 0.2	5.2 ± 0.1
18-hydroxy-9,10-epoxyoctadecanoic acid	22.5 ± 0.2	57.2 ± 0.4	62.3 ± 0.4
unknown monomer (peak H)	31.7 ± 0.3	30.3 ± 0.3	19.9 ± 0.2
9,16-dihydroxyhexadecanoic acid	1.9 ± 0.1	0	0
10,16-dihydroxyhexadecanoic acid	30.5 ± 0.3	3.0 ± 0.1	3.3 ± 0.1
9,10,18-trihydroxyoctadecanoic acid	0.4 ± 0.1	2.0 ± 0.1	9.3 ± 0.2

[a] Values shown are the means ± SD from five experiments (total of 50 membranes).

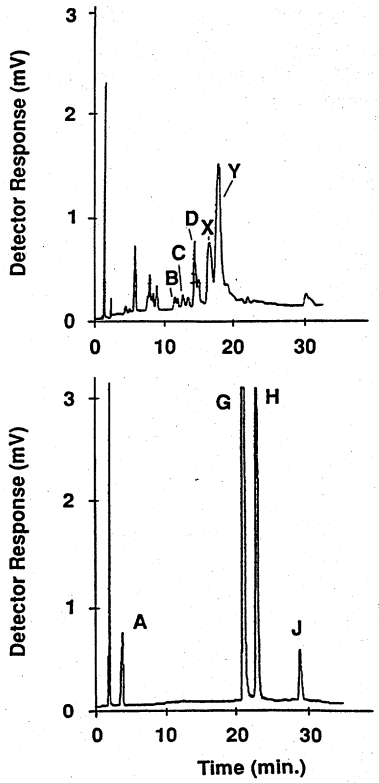

Figure 2. UV (a, top) and ELSD (b, bottom) chromatogram of a cutinase hydrolysate of a NY 10355 pear leaf cuticular membrane. X and Y are additional unknown components. Lettered peaks are identified in Table 1, and labels are split between the top and bottom panels.

10,16-dihydroxyhexadecanoic acid comprised the greatest relative amounts of the fatty acids released and were of equal values even though the latter constituted a much greater percentage of the fatty acids initially present on the basis of chemical hydrolysis (Table 1). Typical UV and ELSD chromatograms of a cutinase hydrolysate of a cuticular membrane from NY 10355 leaves are shown in Figure 2. For NY 10355 and Erabasma, the major fatty acid released was 18-hydroxy-9,10-epoxyoctadecanoic acid, even though the results of base hydrolysis indicated that this fatty acid is a relatively minor component of the cuticular membranes (Table 1). Hydrolysis of pear cuticular membranes with cutinase from *Nectria* led to the release of small amounts of vanillic, *p*-coumaric, and ferulic acids and two major UV-absorbing unknown molecules (labeled X and Y) (Figure 2). To determine whether these unknowns were oligomers, the peak fractions were collected and treated with methanolic KOH. Since KOH treatment did not change the retention times of these peaks, it appears unlikely that they are oligomers. The unknown compound eluting at 23 min as detected by ELSD appears to be identical to the unknown (designated compound H) shown in Figure 1b on the basis of identical retention times.

In summary, the monomeric composition of dewaxed pear leaf cuticular membranes (primarily cutin with residual cuticular wax) was defined with 10,16-dihydroxyhexadecanoic acid as the major cutin monomer present. Pear genotypes exhibited quantitative differences in monomer composition, with the pear psylla resistant genotype NY 10355 having significantly higher levels of 16-hydroxyhexadecanoic acid compared with the two more susceptible genotypes Bartlett and Erabasma. Tree-to-tree variability in monomer composition was demonstrated using leaves of Erabasma. On the basis of the weights of chloroform/methanol-soluble monomeric material, the cuticular membranes of NY 10355 are thinner than the membranes of the other two genotypes. Finally, differences in susceptibility to digestion with lipases and a cutinase indicated that there are structural differences among the membranes of the three pear genotypes. The determination of whether any of the differences noted in this study are involved in resistance to attack to pear psylla will require further study.

LITERATURE CITED

Baker, E. A. Chemistry and Morphology of Plant Epicuticular Waxes. In *The Plant Cuticle*; Cutler, F. D., Alvin, K. L., Price, C. E., Eds.; Academic Press: London, 1982.

Baker, E. A.; Procopiou, J. The Cuticles of *Citrus* Species. Composition of the Intracuticular Lipids of Leaves and Fruits. *J. Sci. Food Agric.* **1975**, *26*, 1347–1352.

Bell, R. L.; Stuart, L. C. Resistance in Eastern European *Pyrus* Germplasm to Pear Psylla Nymphal Feeding. *HortScience* **1990**, *25*, 789–791.

Bligh, E. G.; Dyer, W. J. A Rapid Method of Total Lipid Extraction and Purification. *Can. J. Biochem. Physiol.* **1959**, *37*, 911–916.

Bradford, M. M. A Rapid and Sensitive Method for the Quantitaion of Microgram Quantities of Protein Utilizing the Principle of Protein–Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.

Butt, B. A.; Stuart, L. C. Oviposition by Summer and Winter Forms of Pear Psylla (Homoptera: Psyllidae) on Dormant Pear Budwood. *Environ. Entomol.* **1985**, *15*, 1109–1110.

Butt, B. A.; Stuart, L. C.; Bell, R. L. Feeding Behavior of Pear Psylla (Homoptera: Psyllidae) Nymphs on Susceptible and Resistant *Pyrus* Germplasm. *J. Econ. Entomol.* **1988**, *81*, 1394–1397.

Challice, J. S.; Williams, A. H. Phenolic Compounds of the Genus *Pyrus*-I. The Occurrence of Flavones and Phenolic Acid Derivatives of 3,4-Dihydroxybenzyl Alcohol-4-Glucoside in *Pyrus calleryana*. *Phytochemistry* **1968**, *25*, 119–130.

Chapman, R. F.; Bernays, E. A. Insect Behavior at the Leaf Surface and Learning as Aspects of Host Plant Selection. *Experientia* **1989**, *45*, 215–222.

Classen, D.; Arnason, J. T.; Serratos, J. A.; Lambert, J. D. H.; Nozzolillo, C.; Philogene, B. J. R. Correlation of Phenolic Acid Content of Maize to Resistance to *Sitophilus zeamais*, the Maize Weevil, in CIMMYT'S Collections. *J. Chem. Ecol.* **1990**, *16*, 301–315.

Eigenbrode, S. D.; Espelie, K. E.; Shelton, A. M. Behavior of Neonate Diamondback Moth Larvae (*Plutella xylostella* L.) on Leaves and on Extracted Leaf Waxes of Resistant and Susceptible Cabbages. *J. Chem. Ecol.* **1991**, *17*, 1691–1704.

Gerard, H. C.; Moreau, R. A.; Fett, W. F.; Osman, S. F. Separation and Quantitation of Hydroxy and Epoxy Hydroxy Fatty Acids by High-Performance Liquid Chromatography with an Evaporative Light-Scattering Detector. *J. Am. Oil Chem. Soc.* **1992a**, *69*, 301–304.

Gerard, H. C.; Osman, S. F.; Fett, W. F.; Moreau, R. A. Separation, Identification and Quantification of Monomers from Cutin Polymers by High-Performance Liquid Chromatography and Evaporative Light-Scattering Detector. *Phytochem. Anal.* **1992b**, *3*, 139–144.

Gerard, H. C.; Fett, W. F.; Osman, S. F.; Moreau, R. A. Evaluation of Cutinase Activity of Various Industrial Lipases. *Biotechnol. Appl. Microbiol.* **1993**, *17*, 181–189.

Gilbert, E. J. *Pseudomonas* Lipases: Biochemical Properties and Molecular Cloning. *Enzyme Microb. Technol.* **1993**, *15*, 634–645.

Hankin, L.; Kolattukudy, P. E. Metabolism of a Plant Wax Paraffin (*n*-nonacosane) by a Soil Bacterium (*Micrococcus cerificans*) *J. Gen. Microbiol.* **1968**, *51*, 457–463.

Holloway, P. J. The Chemical Composition of Plant Cutins. In *The Plant Cuticle*; Cutler, F. D., Alvin, K. L., Price, C. E., Eds.; Academic Press: London, 1982.

Klingauf, F. The action of the glycoside phlorizin on the host selection behavior of *Rhapalosiphum insertum* (Wald.) and *Aphis pomi* de Geer (Homoptera: Aphididae). *Z. Angew. Entomol.* **1971**, *68*, 41–55.

Kolattukudy, P. E. Lipid-Derived Defensive Polymers and Waxes and Their Role in Plant-Microbe Interaction. In *The Biochemistry of Plants: A Comprehensive Treatise, Vol. 9, Lipids Structure and Function*; Stumpf, P. K., Conn, E. E., Eds.; Academic Press: New York, 1987.

Kolattukudy, P. E.; Purdy, R. E.; Maiti, B. Cutinases from Fungi and Pollen. *Methods Enzymol.* **1981**, *71*, 652–664.

Miller, R. L.; Bills, D. D.; Buttery, R. G. Volatile Components from Bartlett and Bradford Pear Leaves. *J. Agric. Food Chem.* **1989**, *37*, 1476–1479.

Riley, R. G.; Kolattukudy, P. E. Evidence for Covalently Attached *p*-Coumaric Acid and Ferulic Acid in Cutins and Suberins. *Plant Physiol.* **1975**, *56*, 650–654.

Schalk, J. M.; Ratcliffe, R. H. Evaluation of ARS Program on Alternative Methods of Insect Control; Host Plant Resistance to Insects. *Bull. Entomol. Soc. Am.* **1976**, *22*, 7–10.

Walton, T. J.; Kolattukudy, P. E. Determination of the Structures of Cutin Monomers by a Novel Depolymerization Procedure and Combined Gas Chromatography and Mass Spectrometry. *Biochemistry* **1972**, *11*, 1885–1897.

Woodhead, S.; Chapman, R. F. Insect Behavior and the Chemistry of Plant Surface Waxes. In *Insects and the Plant Surface*; Juniper, B., Southwood, Sir R., Eds.; Arnold: London, 1986.

Yang, G.; Espelie, K. E.; Todd, J. W.; Culbreath, A. K.; Pittman, R. N.; Demski, J. W. Cuticular Lipids from Wild and Cultivated Peanuts and the Relative Resistance of These Peanut Species to Fall Armyworm and Thrips. *J. Agric. Food Chem.* **1993**, *41*, 814–818.

Received for review May 10, 1993. Revised manuscript received September 29, 1993. Accepted October 12, 1993.[⊗] Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1993.